

| Constructs |  | Deletion<br>(nucleotides) | pABV<br>number | M-<br>expression | N-<br>expression |
|------------|--|---------------------------|----------------|------------------|------------------|
|            |  | -                         | 437            | +                | +                |
|            |  | Δ 11788-14139             | 594            | -                | +                |
|            |  | Δ 14585-14984             | 521            | - <sup>1)</sup>  | -                |
|            |  | Δ 11788-14584             | 664            | -                | +                |
|            |  | Δ 14985-15111             | 668            | - <sup>1)</sup>  | -                |

<sup>1)</sup> Identical results were obtained in IPMA using MAbs against GP<sub>3</sub> and GP<sub>4</sub>

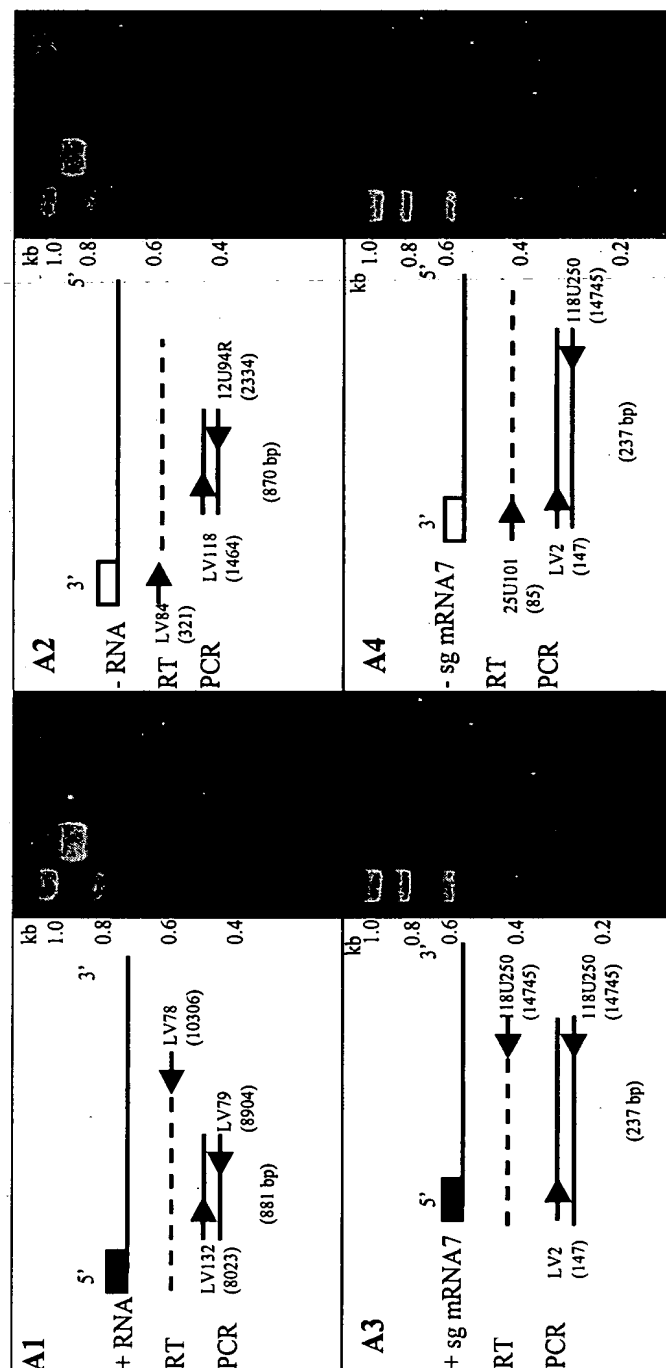
Fig. 1A

| Constructs | Deletion (nucleotides) | PABV number | M-expression    |
|------------|------------------------|-------------|-----------------|
|            | -                      | 437         | + <sup>1)</sup> |
|            | Δ 14588-14936          | 605         | -               |
|            | Δ 14588-14885          | 604         | -               |
|            | Δ 14588-14786          | 603         | -               |
|            | Δ 14588-14687          | 602         | -               |
|            | Δ 14588-14642          | 624         | +               |
|            | Δ 14599-14642          | 625         | +               |
|            | Δ 14588-14600          | 626         | + <sup>1)</sup> |
|            | Δ 14938-14980          | 638         | + <sup>1)</sup> |
|            | Δ 14887-14980          | 637         | +               |
|            | Δ 14788-14980          | 636         | +               |
|            | Δ 14686-14980          | 635         | +               |
|            | Δ 14643-14686          | 631         | -               |
|            | Δ 14643-14676          | 632         | -               |
|            | Δ 14643-14664          | 633         | -               |
|            | Δ 14643-14652          | 634         | +               |
|            | Δ 14653-14686          | 696         | -               |
|            | rescue of 696          | 730         | + <sup>1)</sup> |

<sup>1)</sup> Identical results were obtained in IPMA using MAbs 122.17 against N

Fig. 1B

Fig. 2



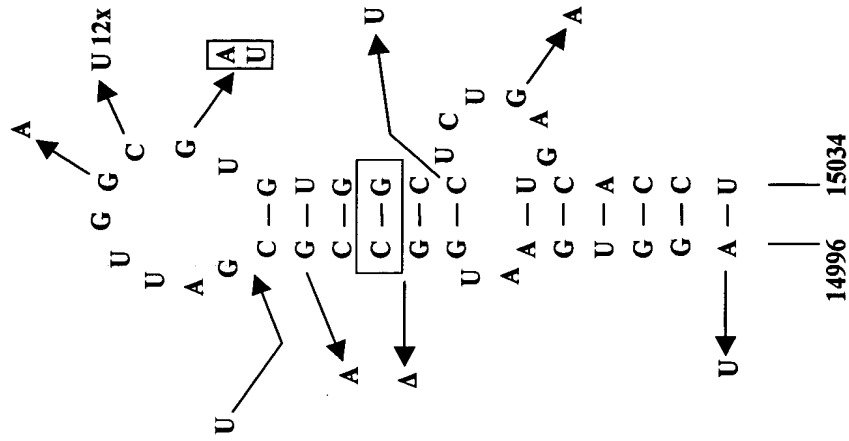


Fig. 3B

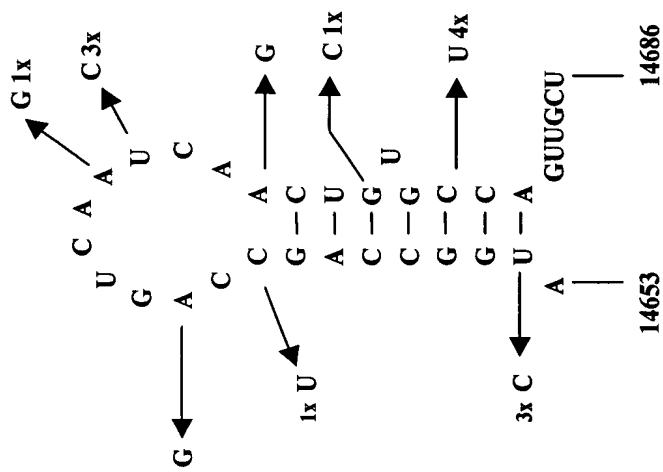


Fig. 3A



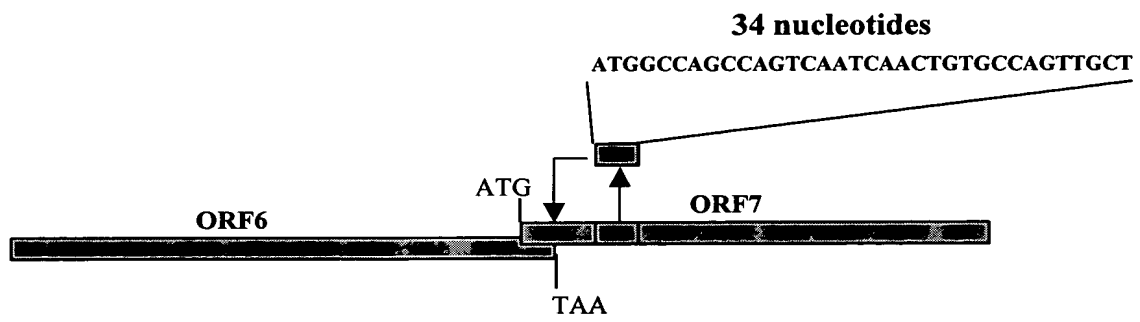


Fig. 5

A

```
LV      : MAGKNQSOKKKKSTAPMNGQPVNQLCOLLGAMIKSORQ---QPRGGQAKKKKPEKPHFPLAAEDDIRHH : 67
VR2332 : MPNNNGKQQRK-----KGDGQPVNQLCQMLGKIIAQONQSRGKGPCKKNKKNPEKPHFPLATEDDDVRHH : 66
      * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
LV      : LTQTERSLCLQSIQTAFTNQAGTASLSSSGKVSFQVEFMLPVAHTVRLIRVTSTASQGAS : 128
VR2332 : FTPSERQLCLSSIQTAFTNQAGTCTLSDSGRISYTVFESLPTHHTVRLIRVTASPSA---- : 123
      * ** *** ***** ** ** * ** ** * *****
```

B

```
LV      : TTAAACAGTCA-----GGTGAATGCCCGCGATTGGCG : 32
VR2332 : TGGGCTGGCATTCTTGAGGCATCTCAGTGTTTGAATTGGAAGAAATGTGTGGTGAATGGCACTGATTGACA : 70
      * * * * * ***** *
LV      : TGTGGCCTCTGAGTCACCTATTCAATTAGGGCGATCACATGGGGGTCACTTAATCAGGCAGGAACCAT : 102
VR2332 : TTGTGCCTCTAAGTCACCTATTCAATTAGGGCGACCGTGTGGGGGTGAGATTTAATT-GGCGAGAACCAT : 139
      * ***** ***** * * ***** * * *****
LV      : GTGACCGAAATTAAAAAAA: 122
VR2332 : GCGGCCGAAATTAAAAAAA: 159
      * * *****
```

Fig. 6








| Constructs  | Deletion<br>(nucleotides /<br>amino acids) | Plasmid<br>number | M-<br>expression | N-<br>expression | Virus<br>production |
|---|--|-------------------|------------------|------------------|---------------------|
|    | wild type                                  | 437               | +                | +                | +                   |
|    | $\Delta 14975-14980$ / $\Delta 2$          | 639               | +                | +                | +                   |
|    | $\Delta 14969-14980$ / $\Delta 4$          | 694               | +                | +                | +                   |
|    | $\Delta 14966-14980$ / $\Delta 5$          | 745               | +                | +                | +                   |
|  | $\Delta 14963-14980$ / $\Delta 6$          | 746               | +                | +                | +                   |
|  | $\Delta 14960-14980$ / $\Delta 7$          | 747               | +                | +                | -                   |
|  | $\Delta 14957-14980$ / $\Delta 8$          | 748               | +                | +                | -                   |
|  | $\Delta 14954-14980$ / $\Delta 9$          | 695               | +                | +                | -                   |
|  | $\Delta 14989-14995$                       | 693               | +                | +                | +                   |
|  | $\Delta 14989-15020$                       | 729               | -                | -                | -                   |

Fig. 7



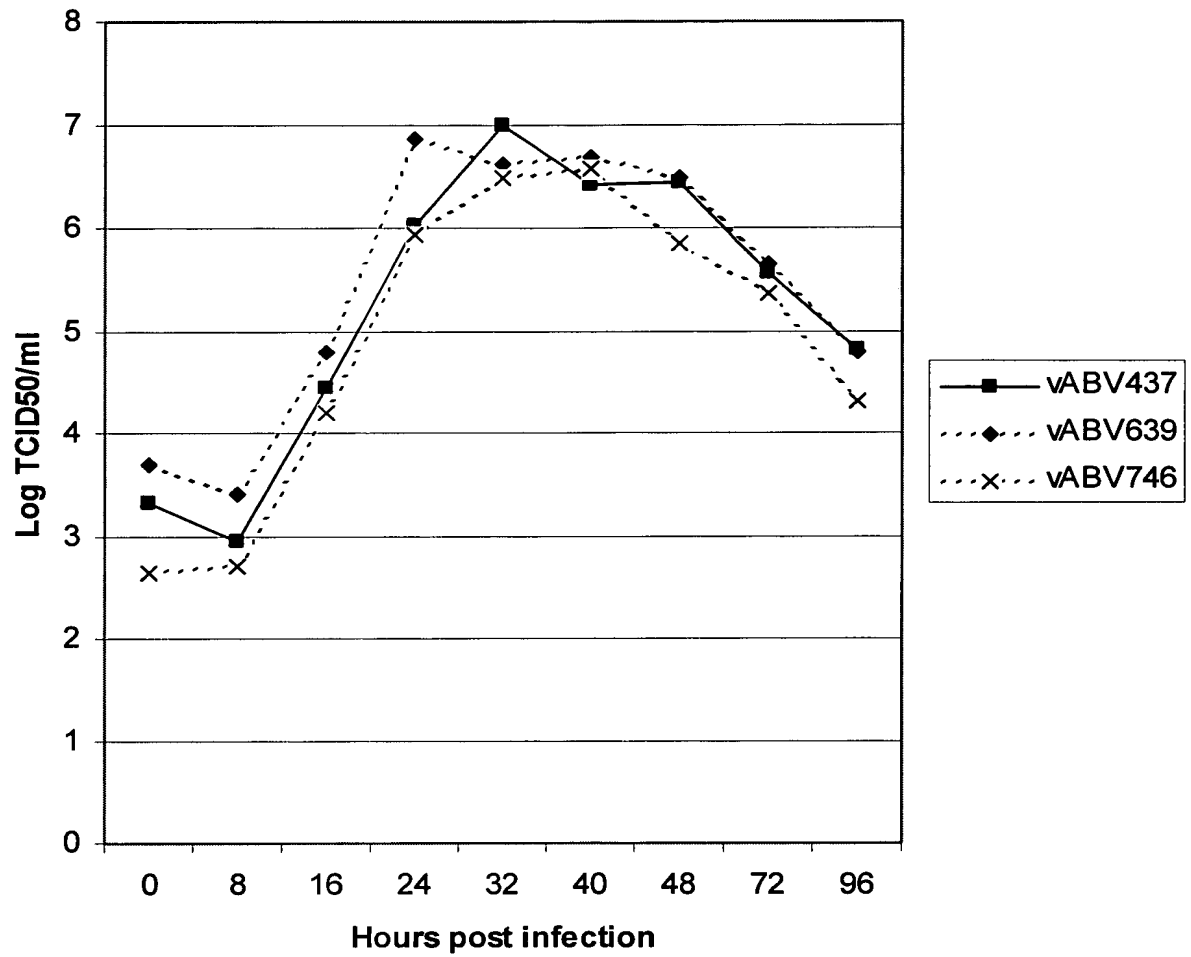
**Growth curves of PRRSV deletion mutants**

Fig. 8

Fig. 9

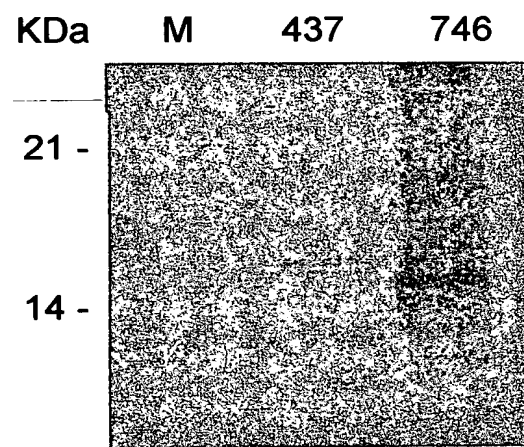


Fig. 10

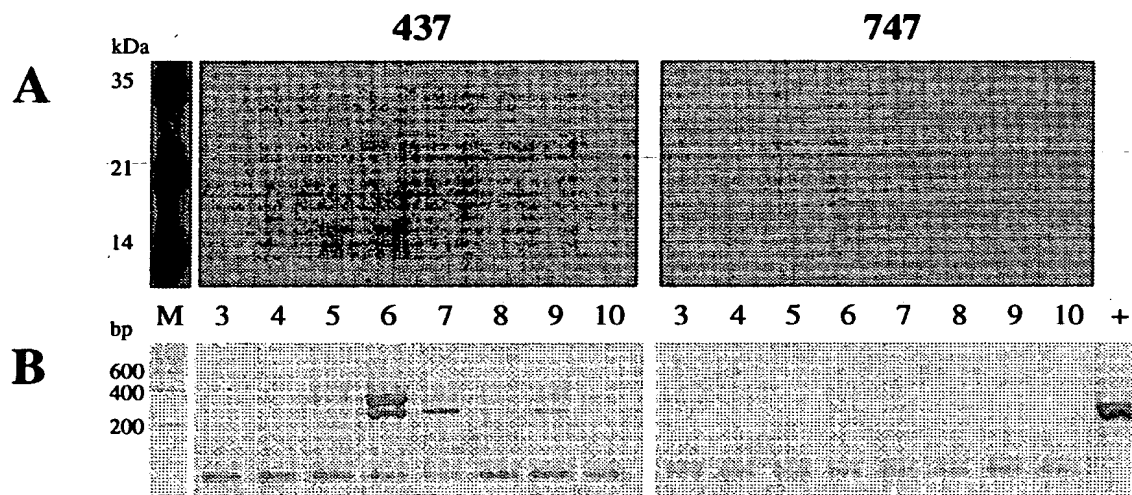


TABLE 1: Sequences of the primers used to introduce deletions by PCR, and primers used to sequence the introduced mutations.

| Primer   | Sequence of the primer <sup>a</sup>                 | Orientation | Purpose (pABV) | Location |
|----------|---|-------------|----------------|----------|
| 119R218R | 5' ATGACATCCGGCACCACC 3'                            | +           | Sequencing     | 14782    |
| LV20     | 5' CCTGATTAAAGCTTGACCCC 3'                          | -           | Sequencing     | 15066    |
| LV75     | 5' TCTAGGAATCTAGACGATCG 3'                          | -           | XbaI -site     | 15088    |
| LV155    | 5' ACGTGCCTTAACTCGTCAAGTATGGCCGGTAAAAACAGAGCCAGA 3' | +           | HpaI-site      | 14582    |
| LV204    | 5' ACGTGCCTTAACTTAACTTGACTGGCGGATGTAGA 3'           | -           | 639            | 14974    |
| LV213    | 5' TGCAAGTTAATTAAAGGTGAATGGCCGCGA 3'                | +           | 693            | 14996    |
| LV 214   | 5' GACTGTTTAAATTAACCTGGCGGATGTA 3'                  | -           | 694            | 14958    |
| LV215    | 5' GACTGTTTAAATTAAGTCACGCGAATC 3'                   | -           | 695            | 14942    |
| LV239    | 5' TGCAAGTTAATTAAAGCCTCTGAGTCA 3'                   | +           | 729            | 15021    |
| LV263    | 5' GACTGTTTAAATTAAGCGGATGTAGA 3'                    | -           | 745            | 14954    |
| LV264    | 5' GACTGTTTAAATTAAGATGTAGAAGTC 3'                   | -           | 746            | 14951    |
| LV265    | 5' GACTGTTTAAATTAAGTAGAAGTCACG 3'                   | -           | 747            | 14948    |
| LV266    | 5' GACTGTTTAAATTAAGAAAGTCACGCGA 3'                  | -           | 748            | 14945    |

<sup>a</sup> The restriction sites are underlined.

Fig. 11

Fig. 12

TABLE 1: Sequences of the primers used to introduce deletions by PCR, primers used to sequence the introduced mutations, and primers used for the strand-specific RT-PCR

| Primer  | Sequence of the primer <sup>a</sup>                          | Orien-<br>tation | Purpose<br>(pABV)           | Location |
|---------|--|------------------|-----------------------------|----------|
| L18U250 | 5' CAGCCAGGGGAAAAATGTGGC 3'                                  | -                | Sequencing / Strand-sp. PCR | 14745    |
| L2U94R  | 5' CACCTGTACCTGCTCATGT 3'                                    | -                | Strand-sp. PCR              | 2334     |
| 25U101  | 5' GTTCTAGCCCAACAGGTATC 3'                                   | +                | Strand-sp. RT               | 85       |
| LV2     | 5' AGCGGGAAGGATCCACCGAGTAT 3'                                | +                | Strand-sp. PCR              | 147      |
| LV17    | 5' CCTTTGACGAGCTCTCCGC 3'                                    | +                | Sequencing                  | 14045    |
| LV20    | 5' CTTGATTAAAAGCTTGACCCC 3'                                  | +                | Sequencing                  | 15066    |
| LV75    | 5' TCTAGGAATCTAGACGATCG                                      | -                | PCR <i>Xba</i> I -site      | 15088    |
| LV76    | 5' TCTAGGAATCTAGACGATCG(T40) 3'                              | -                | RT                          | 15088    |
| LV78    | 5' CCTGGGATGAATCTATGGT 3'                                    | -                | Strand-sp. RT               | 10306    |
| LV79    | 5' GACAAGATCATCAGAGTATACC 3'                                 | -                | Strand-sp. PCR              | 8904     |
| LV84    | 5' AGAGCTTCAGGACACTGACC 3'                                   | +                | Strand-sp. RT               | 321      |
| LV112   | 5' CATTTCACCTGACTGTTTAAATTAACCTTGACCCCTGA 3'                 | -                | PCR <i>Pae</i> I -site      | 14981    |
| LV118   | 5' TTACCACTACTCTCCACCG 3'                                    | +                | Strand-sp. PCR              | 1464     |
| LV132   | 5' CCTACTGTCCCTATAGTTC 3'                                    | +                | Strand-sp. PCR              | 8023     |
| LV151   | 5' ACCAGAGCAGAGAAAAAGTACAGCTGGGTGCAATGAT 3'                  | +                | PCR (631)                   | 14611    |
| LV152   | 5' ACCAGAGCAGAGAAAAAGTACAGCTGCCAGTTGCTGG 3'                  | +                | PCR (632)                   | 14611    |
| LV153   | 5' ACCAGAGCAGAGAAAAAGTACAGCTTCAATCAACTGT 3'                  | +                | PCR (633)                   | 14611    |
| LV154   | 5' ACCAGAGCAGAGAAAAAGTACAGCTATGGCCAGCCAG 3'                  | +                | PCR (634)                   | 14611    |
| LV155   | 5' ACGTGGTTAACTCGTCAAGTATGGCCGTAAAAACCCAGAGCCAGA 3'          | +                | <i>Hpa</i> I-site PCR       | 14582    |
| LV188   | 5' ACGTGGTTAACTAAAGGTGCAATGATAAAGTCCCA 3'                    | +                | PCR (602)                   | 14582    |
| LV189   | 5' ACGTGGTTAACTAAATCCGGCACCACCTCACCCA 3'                     | +                | PCR (603)                   | 14582    |
| LV190   | 5' ACGTGGTTAACTAAAGGGAAGGTCACTTTTCAGGT 3'                    | +                | PCR (604)                   | 14582    |
| LV191   | 5' ACGTGGTTAACTAACGCCCTGATTCGGTGACTTC 3'                     | +                | PCR (605)                   | 14582    |
| LV195   | 5' ACGTGGTTAACTAACCCGATGGGGAATGGCCAG 3'                      | +                | PCR (624)                   | 14582    |
| LV196   | 5' GGAGTGGTTAACTCGTCAAGTAAACCGATGGGGAATGGCCAG 3'             | +                | PCR (625)                   | 14582    |
| LV197   | 5' ACGTGGTTAAACGCCCGGTAAAAACCCAGAGC 3'                       | +                | PCR (626)                   | 14582    |
| LV198   | 5' GCTCGTGTAGCTTTAGCATCACATACAC 3'                           | +                | <i>Nhe</i> I -site PCR      | 14140    |
| LV200   | 5' ACGTGGTTAAATTAACCCAGCAACTGGCAGATTG 3'                     | -                | PCR (635)                   | 14981    |
| LV201   | 5' ACGTGGTTAAATTAATGTATCTTCAGCAGCCAG 3'                      | -                | PCR (636)                   | 14981    |
| LV202   | 5' ACGTGGTTAAATTAACCCGTGGATGAAAGCGACGC 3'                    | -                | PCR (637)                   | 14981    |
| LV203   | 5' ACGTGGTTAAATTAACCCGACTGATGACCAACCCG 3'                    | -                | PCR (638)                   | 14981    |
| LV204   | 5' ACGTGGTTAAATTAACCTTGACTGGCGATGTAGA 3'                     | -                | PCR (639)                   | 14981    |
| LV216   | 5' ACCAGAGCAGAGAAAAAGTACAGCTCCGATGGGGAG<br>GGTCAATGAT 3'     | +                | PCR (696)                   | 14611    |
| LV268   | 5' ACCAGAGCAGAGAAAAAGTACAGCTCCGATGGGGA 3'                    | +                | PCR (769)                   | 14611    |
| LV269   | 5' CTCGATGGGAATGGCCAGCCAGTGTAGAACTGTGCCAGT 3'                | +                | PCR (769)                   | 14641    |
| LV270   | 5' TGCAAGTTAAITTAACACAGTCAGGTGAATGGCCGCTAACCGGTGTGGCCTC 3' + | +                | PCR (768)                   | 14981    |

<sup>a</sup> The restriction sites are underlined.